though it is unnecessary), the frequency of occurrence of a profile can be estimated by limiting the statistic to the number of individuals in the data base, at each particular locus, carrying the same profile as the evidentiary material. Since there is no evidence of linkage disequilibrium (i.e., gametic phase disequilibrium), the frequency of each observed profile at each locus could be multiplied together to estimate the occurrence of a multilocus phenotype. Using either the assumption of HWE or observed phenotypes would in actuality result in no forensically significant differences in the final estimate.

Fifth, a critical discussion of Dr. Green's proposed two-allele-per-locus approach is in order. When an approach for forensic analysis is considered, it is essential to assess the ability of the system to resolve issues peculiar to forensics. During the act of violent crimes, it is common for body fluid/tissues from different contributors to mix. Use of a two-allele system would make it more difficult to resolve whether one or more individuals have contributed to the evidentiary material. For instance, an apparent heterozygote in some cases potentially could be a composite of two homozygotes (or a heterozygote and a homozygote). Obviously, the elucidation of multiple contributors can be more readily accomplished with highly polymorphic systems.

In conclusion, it is hoped that this discussion has been helpful in further addressing the issues surrounding the application of population genetic principles to forensic situations. Through the discovery process, the FBI population data have been made available to many investigators; while many have chosen to present hypotheticals regarding potential population genetics concerns, Chakraborty (1991), Chakraborty et al. (in press), Chakraborty and Jin (submitted), Weir (submitted), and B. Devlin (personal communication) actually performed analyses to evaluate the effects of HWE and gametic phase disequilibrium. Both the data and theory support the conclusion that the frequencies of VNTR profiles can be estimated as the products of the frequencies of the constituent elements. In addition, highly polymorphic loci are the most informative loci both for discriminating between individuals and for evaluating whether a biological evidentiary sample may be composed of material from one or more individuals.

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## Molecular Genetic Analysis of a Sporadic Case of Leber Hereditary Optic Neuropathy

*To the Editor:*

Rapid progress has been made in understanding the etiology of the mitochondrial genetic disease Leber hereditary optic neuropathy (LHON). Affected individuals show a sudden, usually painless, bilateral loss of central vision generally accompanied by characteristic retinal vascular changes; the resulting central scotoma are usually permanent (Nikoskelainen 1985; Newman et al. 1991). The risk of developing LHON is strictly maternally inherited with incomplete penetrance: as a general rule, about 50% of the males and 10% of the females are affected (Lundsgard 1944; van Senus 1963; Seedorff 1970; Nikoskelainen et al. 1987). Furthermore, there are several other causes of

bilateral optic neuropathy, and, until recently, a firm diagnosis of LHON required either the observation of the patient during the acute phase or a previous history of the disease in maternally related family members.

Diagnosis of LHON was placed on much more solid footing when the disease was associated with a mutation at nucleotide 11778 of the mtDNA (Wallace et al. 1988; Singh et al. 1989). This sequence change results in the substitution of histidine for arginine at amino position 340 of the ND4 protein, one of the seven mitochondrially encoded subunits of complex I (NADH–ubiquinone oxidoreductase). This mutation is found in 50%–70% of all LHON pedigrees (Holt et al. 1989; Vilkki et al. 1990; Poulton et al., in press). More recently, the primary mitochondrial gene mutation in a large proportion of non-ND4 LHON pedigrees has been identified as a transition, at nucleotide 3460, which results in the substitution of threonine for alanine at position 52 of the ND1 protein. This mutation has been found in seven English/Australian (Howell et al. 1991*a*) and three Finnish (Huoponen et al. 1991) LHON pedigrees. We have estimated that the ND1 mutation is the primary etiologic event in 15%–25% of all LHON pedigrees (Howell et al. 1991*a*).

We have recently completed the molecular genetic analysis of a three-generation Italian family in which there was the sporadic occurrence of bilateral optic neuropathy in a single family member. The results of this study are relevant to the diagnosis of LHON and, on a more basic level, to the inheritance of deleterious mitochondrial gene mutations.

The proband is a 20-year-old male who was noted to have a slight loss of vision in 1989, first in the left eye and then in the right. Loss of vision progressed rapidly in the left eye, with a central scotoma developing within 1 wk. A similar rapidity of vision loss subsequently occurred in the right eye. At present, the patient is able to see only shadows, using his peripheral vision. Since the rapid loss of vision in October 1989, this patient has been examined several times. Noteworthy is the fact that he had no systemic findings and that fundoscopy did not reveal any retinal changes typical of LHON. Thus, peripapillary telangiectasia was not observed during the acute phase. Both analysis of cerebrospinal fluid and a computed-tomography scan were negative. Magnetic resonance imaging indicated an abnormal signal in the left cerebellum, which has not changed subsequently. Blood tests for syphilis, HIV, and other viral infections were negative. Except for poorly reactive pupils and the optic neuropathy, the patient's neurological exam was essentially negative. Although his maternal relatives have not been examined in depth, there is no report of any ophthalmological or neurological abnormalities.

To determine whether this individual carried either the ND4/11778 or ND1/3460 LHON mutations, DNA was isolated from the white blood cell/platelet fraction of a small blood sample by using standard procedures. Fragments of the mitochondrial ND4 and ND1 genes—approximately 300 bp in length—spanning nucleotides 11778 and 3460, respectively, were PCR amplified and cloned into M13 vectors, and the nucleotide sequence was determined as described in previous publications from this laboratory (Howell and McCullough 1990; Howell et al. 1991*b*). It was found that the proband did not carry the ND4/11778 LHON mutation. However, the ND1/3460 mutation was present and, moreover, was apparently homoplasmic, as the sequence change was found in all 45 clones analyzed.

With this information in hand, blood samples were obtained from the proband's father (an internal normal control), mother, younger brother, two maternal aunts, and maternal grandmother. None of these individuals has shown any signs of the optic neuropathy. They were analyzed in a similar fashion for the ND1/3460 mutation, and the results, involving the sequencing analysis of more than 200 independent M13 clones, are summarized in figure 1.

These studies establish clearly that the ND1/3460 mutation is carried in members of the two preceding maternal generations but in a heteroplasmic state with a large proportion of the wild-type allele. The grandmother and the two aunts showed only very low proportions (9% or less) of mitochondrial genomes carrying the mutation; one aunt appeared to have completely lost the mutation through segregation. In contrast, his mother and brother have a higher proportion of the mutant allele (27% and 16%, respectively) but still much less than that in the affected proband. As expected, the proband's father did not carry the ND1/3460 mutation. As discussed in previous publications (Howell et al. 1991*a*, 1991*b*), multiple independent PCR reactions (six to eight in the present study) are pooled so that the marked intrafamilial variation in the frequencies of the mutant allele reflect, with acceptable accuracy, the population value for the white blood cell/platelet fractions.

Heteroplasmy of both the ND4/11778 (Holt et al. 1989; Bolhuis et al. 1990; Lott et al. 1990; Vilkki et al. 1990) and ND1/3460 (Howell et al. 1991*a*)

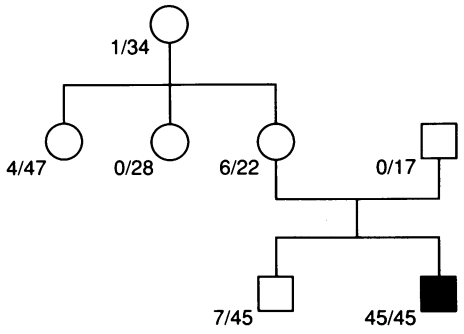


**Figure 1** Pedigree of Italian LHON family. The fractions to the left of the symbols indicate the number of mutant alleles as a function of the total number of independent clones analyzed by nucleotide sequencing.

mutations has been observed in several LHON pedigrees. However, this family is atypical in showing such extreme variation in allele frequencies. One similar example has appeared in the literature. Vilkki et al. (1990) have described a small Finnish LHON family in which there is a single affected individual. In generation III, the frequency of the ND4/11778 mutant allele varied from 0% to 100% among siblings; the mutant-allele frequency for their mother, unfortunately, was not ascertainable. Female III-1 had two sons, both homoplasmic for the 11778 mutation and one of whom was affected with the optic neuropathy. As a technical point, the study by Villki et al. (1990) was carried out by analyzing PCR-amplified DNA for the presence (wild-type allele) or absence (11778 mutation) of a diagnostic *Sfa*N1 restriction site. In our experience, this approach is not completely reliable, as cleavage with this restriction enzyme is occasionally incomplete (N. Howell, unpublished data).

The rapid segregation of mitochondrial genotypes in mammals is the experimental basis underlying the bottleneck hypothesis, in which it is posited that the number of segregating mtDNA molecules is reduced to a small number (20–100) at one stage of oogenesis (Ashley et al. 1989). The rapid segregation of the ND1/3460 mutation in this LHON family would be another set of results supporting this hypothesis. However, in other families, segregation of mitochondrial genotypes do *not* appear to support this hypothesis (N. Howell, unpublished data).

Even in LHON families in which the primary mitochondrial gene mutation is apparently homoplasmic, the optic neuropathy shows a low penetrance (discussed in Howell et al. 1991*b*). The mechanism underlying this phenomenon is unknown at present. This uncertainty notwithstanding, we conclude that the sporadic nature of the LHON in this family is due to presence of only one individual in whom the frequency of the mutant allele is sufficiently high to have produced a significant risk of developing the optic neuropathy. Nikoskelainen et al. (1988) have demonstrated the value, for the diagnosis of LHON, of examining unaffected maternal relatives for peripapillary microangiography in isolated cases of bilateral optic neuropathy. However, this is not always feasible. Furthermore, a negative finding for the microangiopathy is not conclusive, since about two-thirds of the unaffected relatives in LHON families do not display this ophthalmological change (Nikoskelainen et al. 1988). The results presented here, therefore, should be a stimulus to the analysis of other sporadic cases of bilateral optic neuropathy to determine whether the affected individual has LHON as defined by the presence of the ND4/11778 or ND1/3460 mitochondrial gene mutations.

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## Leber Optic Neuropathy

*To the Editor:*

I am writing regarding two articles published in the *Journal* (Howell and McCullough 1990; Howell et al. 1991). Both articles report studies of the mtDNA of a large Queensland family with a disorder described as Leber hereditary optic neuropathy (LHON). In the introduction to both articles, the authors note that there is a "bilateral retinal degeneration." I believe that the authors are confusing LHON with Leber congenital amaurosis. Leber congenital amaurosis is indeed a retinal degeneration with an autosomal recessive disorder; the disease may be heterogeneous, and the mutation(s) are unknown. LHON is an optic nerve disorder characterized by acquired, rather than congenital, visual impairment and is believed to be a mitochondrial genetic disease.

In addition to the above confusion, the readers of the *Journal* should be made aware of the fact that individuals with LHON do not have an encephalopathy. The studies of this family are interesting, and the authors provide convincing evidence for a mitochondrial defect(s) as the basis for the disease. However, in view of the atypical clinical features of the disease in this family, it might be advantageous to describe the condition as a LHON-*like* disorder.

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